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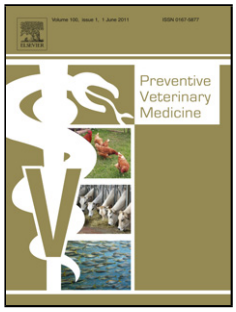

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Short Communication

A case of low success of blind vaccination campaigns against myxomatosis and rabbit haemorrhagic disease on survival of adult European wild rabbits

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Highlights

- Vaccination campaigns against myxomatosis and rabbit haemorrhagic disease (RHD) are commonly used in translocation programs
- For economic and logistic reasons rabbits are vaccinated without previously assessing their immunological status (i.e. blind vaccination campaigns)
- We tested the efficacy of blind vaccination based on rabbit survival in three enclosures where wild rabbits were kept in semi-natural conditions
- Average monthly survival of vaccinated rabbits did not differ to that of unvaccinated individuals
- Blind vaccination may be mostly ineffective and costly since the adults' prevalence of natural antibodies against these endemic diseases is often expected to be high.

A B S T R A C T

Vaccination campaigns against myxomatosis and rabbit haemorrhagic disease (RHD) are commonly used in translocation programs conducted for the purpose of recovering wild European rabbit populations in Iberian Mediterranean ecosystems. In most cases rabbits are vaccinated 'blind' (i.e. without assessing their prior immunological status) for economic and logistic reasons. However there is conflicting evidence on the effectiveness of such an approach. We tested whether blind vaccination against myxomatosis and rabbit haemorrhagic disease improved rabbit survival in a rabbit translocation program where wild rabbits were kept in semi-natural conditions in three enclosures. We conducted nine capture sessions over two years (2008–2010) and used the information collected to compare the survival of vaccinated (n = 511) versus unvaccinated (n = 161) adult wild rabbits using capture-mark-recapture analysis. Average monthly survival was no different for vaccinated versus unvaccinated individuals, both in the period between release and first capture (short-term) and after the first capture onward (long-term). Rabbit survival was lower in the short term than in

the long term regardless of whether rabbits were vaccinated or not. Lower survival in the short-term could be due to the stress induced by the translocation process itself (e.g. handling stress). However, we did not find any overall effect of vaccination on survival which could be explained by two non-exclusive reasons. First, interference of the vaccine with the natural antibodies in the donor population. Due to donor populations have high density of rabbits with, likely, high prevalence of antibodies as a result of previous natural exposure to these diseases. Second, the lack of severe outbreaks during the study period. Based on our findings we argue that blind vaccination of adult rabbits in translocation programs may be often mostly ineffective and unnecessarily costly.
In particular, since outbreaks are hard to predict and vaccination of rabbits with natural antibodies is ineffective, it is crucial to assess the immunological status of the donor population before translocating adult rabbits.

Key words: mortality, *Oryctolagus cuniculus*, translocation, disease control, wildlife management

1. Introduction

In Iberian Mediterranean ecosystems, the European rabbit (*Oryctolagus cuniculus*) is a keystone species that has declined dramatically, with profound implications for conservation and management (Delibes-Mateos et al., 2008). The appearance of myxomatosis in the 1950s and the arrival of rabbit haemorrhagic disease (RHD) at the end of the 1980s caused substantial reductions in rabbit population density (Calvete et al., 2002), and the extinction of many local wild populations (Villafuerte et al., 1995; Delibes-Mateos et al., 2009). Myxomatosis and RHD are caused by a leporipoxvirus and a calicivirus respectively, with both diseases now endemic in the Iberian Peninsula. Resulting rabbit population declines are ongoing; for example a new variant of RHD (i.e. RHDV2) caused a considerable decrease in wild rabbit numbers in France (2010), Spain (2011) and Portugal (2012) (Delibes-Mateos et al., 2014; Le Gall-Reculé et al., 2013).

Considerable effort has been made in recent decades to reduce mortality from these diseases by translocating wild rabbits into areas where wild populations are low or

extinct and implementing vaccination campaigns just before release. Indeed, these measures are among the most frequent management actions made to stimulate the recovery of wild rabbit populations in this region (Angulo and Villafuerte, 2003). In most translocation programs, for supposed economic and logistic reasons rabbits are vaccinated without previously assessing their immunological status, and additional information on sex, heath status or age of the individuals for example, are rarely collected (i.e. 'blind' vaccination; Cabezas et al., 2006). For the purposes of this paper, blind' vaccination campaigns are defined as vaccinating animals without prior knowledge of their immunological status.

The effectiveness of blind vaccination against myxomatosis and RHD has been questioned by hunters, conservationists and wildlife managers. Few studies have assessed its effectiveness in improving rabbit survival and population recovery (Cabezas et al., 2006; Calvete et al., 2004a, 2004b), and the results from those are conflicting. Improvements in survival seem to depend on a variety of factors such as handling stress, individual physical condition, previous immunological status, and population and disease dynamics (Calvete et al., 2004b; Ferreira et al., 2014).

The aim of this case study was to evaluate the effectiveness of a blind vaccination campaign against myxomatosis and RHD in improving both short and long-term survival of wild adult rabbits kept in semi-natural conditions as part of translocations conducted for an endangered predators' conservation program in southwest Spain.

2. Material and methods

Ethics statement

Manipulations of all animals reported in this study were in accordance with Spanish and European regulations (Law 32/2007, R.D. 1201/2005 and Council Directive 2010/63/EU).

Study site, vaccination and data collection

The study took place in the southwestern Iberian Peninsula (Hornachuelos Natural Park; 37°49′ N, 5°15′ W; 100–700 m elevational range), where the climate is Mediterranean with hot, dry summers and cool, wet winters. We analysed capture-recapture data collected during ten capture sessions over two years in three enclosures (E1, E2, E3;

about 4 ha each) built as rabbit breeding zones. The enclosures were between 2 and 4 km apart. Each enclosure was surrounded by a 2.5-m high chain-link fence to exclude terrestrial predators (Rouco et al., 2008) and contained 30 regularly distributed artificial warrens. Water and pellet food was supplied *ad libitum*, along with sown grass to increase the availability of fresh food. Each warren was built with a capture device consisting of a wire net fence with metal cage-traps attached to holes in the fence (see Santoro et al. (2014) for a complete description of the study area).

We conducted a standard vaccination campaign for rabbit translocations. Rabbits were captured from wild donor populations by trapping or ferreting. Rabbits released in E1 (in March 2008) and E2 (in April 2008) were from two populations in the municipality of Córdoba (one donor population per enclosure), while those released in E3 (in May 2008) were from a population in Cádiz. Both donor population areas are located in southwestern Spain, about 70 and 160 km from the study area respectively. Individuals were released into enclosures without quarantine periods, but were confined inside warren pens for their first 6 nights (a practice that improves survival; Rouco et al., 2010). All rabbits released within an enclosure were captured using the same methodology and were handled and released under similar conditions.

Randomly selected rabbits were injected subcutaneously just before release into each enclosure with commercial vaccines against myxomatosis (live Shope fribroma virus Mixohipra-FSA, Hipra Laboratory, Gerona, Spain) and RHD (ARVILAP, Ovejero Laboratory, León, Spain), at the doses recommended for domestic rabbits. Only adult rabbits were translocated. Ninety-four males ($n_{vaccinated}$ = 71) and 159 females ($n_{vaccinated}$ = 121) were released into E1; 56 males ($n_{vaccinated}$ = 44) and 81 females ($n_{vaccinated}$ = 71) into E2; 103 males ($n_{vaccinated}$ = 74) and 179 females ($n_{vaccinated}$ = 130) into E3 (Table 1S, see supplementary material).

Capture-recapture data was collected from 9 live-trapping recapture sessions following the release sessions, from March-June 2008 to April 2010. Captures took place on one night only and involved activation of the capture devices at midday, when the rabbits were less active and mostly underground. The following morning, the rabbits trapped inside the cages were counted and handled (i.e. all animals were weighed, sexed and ear-marked with numbered metal tags (Presadom nº3, France). Previous studies have

shown that live-trapping in our study area can capture a large proportion of the rabbit population (i.e. 50–60% of the population) in only one night (see Santoro et al., 2014). All individuals were released at their capture location. Time intervals between capture sessions were unequal (Table 1S, see supplementary material), but this was specifically accounted for in capture-recapture analyses conducted.

Additionally, from August 2008 to November 2009, in order to determine any outbreak of disease, we inspected on a monthly basis each enclosure by walking inside along 20-30 minutes looking for rabbit carcasses. When possible, causes of death were determined by post-mortem examination of rabbit carcasses. Predation was assigned to raptors when evidence including feathers, characteristic tufts of torn-out fur, or remains of long bones were found. However, rabbits assigned to predation could also be scavenged. Diseases was assigned to those rabbits with clear lesions due to myxomatosis and/or RHD. Deaths included in the "other causes" category included those assigned to handling stress or aggression associated with social interactions (Calvete and Estrada 2004; Moreno et al. 2004).

Goodness of fit

Animals born during the study period were excluded from analyses. To estimate survival, and identify its determinants, we performed a capture-recapture analysis which provides accurate estimates even when not all alive marked individuals are recaptured (Lebreton et al. 1992). Before this analysis we used U-CARE 2.3.2 (Choquet et al., 2005) to test the goodness of fit of the Cormack–Jolly–Seber (CJS) model (a fully parameterized model allowing for time variation in both survival and individual capture probabilities). The fit of the CJS model is routinely assessed prior to full analysis since an adequate fit indicates that the data is also valid for the set of candidate models (with less parameters) used for hypotheses-testing. U-CARE also allows testing for specific causes of lack of fit such as trap-dependence (i.e. individual capture probability depending on whether it was captured or not in the previous session). A goodness of fit analysis was performed separately for each population (E1–E3).

Capture-recapture analyses

At all trapping sessions, a number of randomly selected individuals (N_{total}: E1 = 24, E2 = 32 and E3 = 27) were removed just after being captured and translocated into nearby

areas as a part of the ongoing translocation program. Removals were coded in the data sets and do not affect estimation of parameters nor should they bias survival estimates as the removed individuals were chosen randomly.

Due to logistic constraints, capture–recapture sessions were not synchronised among the three enclosures (E1–E3) (Table 1S, see supplementary material). Furthermore, each enclosure differed in donor population, stocking date and population size. We thus modeled the data from each enclosure separately. Survival was estimated as a monthly rate; for each enclosure we estimated and tested the effect on survival rate of: (i) vaccination status (vaccinated vs. unvaccinated), (ii) sex, and (iii) short-term versus long term effects. Short-term refers to survival between initial release and the first recapture session (e.g. that might be affected by translocation stress), and long-term refers to average survival from the first recapture session to the end of the study. With a focus on survival response to vaccination, we also considered the interaction between vaccination status and time after release (i.e. the short- and long-term survival of vaccinated and unvaccinated rabbits).

Analyses were performed in MARK 7.2 (White and Burnham, 1999). Following the notation used in Lebreton et al. (1992), the global starting model was defined as ($\phi_{vac \times rel \times sex}$ $p_{sex \times time}$) which means that survival (ϕ) is left to vary according to the interaction of vaccination status, short- and long-term from release ($_{rel}$) and sex, while capture probability (p) varies according to sex and time (i.e. different for each capture session). Importantly, fences prevented emigration of individuals from each enclosure. This is important because it allowed us to obtain true survival estimates that uniquely depend on mortality and not on the dispersal process. Model selection of candidate models was based on the Akaike Information Criterion corrected for sample size (AICc; Burnham and Anderson, 2002). If a model accounting for an effect of vaccination appeared to have substantial support (AICc - AICc min $\leq$ 2; Burnham and Anderson, 2002), we used the Likelihood Ratio Test (LRT) against the nested model without vaccination to assess the significance of the vaccination effect.

For each enclosure separately, we first modeled covariates affecting individual probabilities of recapture, and then held the lowest AICc model structure for recapture probability and modeled survival probabilities. Survival estimates were calculated as

averages from the entire set of candidate models, to minimize any potential effect of model selection uncertainty (Burnham and Anderson, 2002).

3. Results

Goodness of fit

No lack of fit was detected for the CJS model for the mark-recapture data from each of the three enclosures (E1: $\chi^2_{[24]} = 18.77$, p = 0.76, Signed Statistic for Trap-Dependence (SSTD) = -1.38, p = 0.17; E2: $\chi^2_{[21]} = 5.07$, p = 0.99, SSTD = -1.23, p = 0.22; E3: $\chi^2_{[40]} = 19.70$, p = 0.99, SSTD = -1.23, p = 0.22).

Capture-recapture estimates

According to the AICc model rankings for enclosures E2 and E3, we cannot discard a positive effect of vaccination on long-term rabbit survival (Table 1, models within 2 ΔAICc are equally plausible). However, this effect was not included in plausible models for enclosure E1, and its magnitude was so small that it was statistically insignificant in all three enclosures (as calculated by Likelihood Ratio Tests; E1, $P_{LRT} = 0.42$; E2, $P_{LRT} = 0.26$; E3, $P_{LRT} = 0.09$). Overall, the probability of rabbit monthly survival was greater in the long-term than it was in the short-term (the period immediately after translocation). We also detected differences in survival between males and females (Table 1), but they varied in magnitude and direction among enclosures (Fig. 1).

Dead rabbits

The causes of death and the number of dead rabbits found during the study period varied between enclosures (Figure 1S, see supplementary material). Out of 167 rabbit carcasses collected during the study period (E1=50, E2=78 and E3=89), 8 in E1 (16%), 33 in E2 (42%) and 22 in E3 (25%) were assigned to diseases. Most of the carcasses were collected during 2009. Those found between August and September 2009 had clear lesions due to myxomatosis, and those found in November 2009 were assigned to RHD.

4. Discussion

Our results revealed that a blind vaccination campaign of adult wild rabbits did not consistently improve their survival. Rather the only consistent difference observed in our study was that rabbit survival was lower in the short term than in the long term

regardless of whether rabbits were vaccinated or not (Fig. 1). This could likely be due to the stress that translocations programs generates to the target species (Teixeira et al., 2007). Survival was also dependent on time period and sex, but with the direction of differences varying among enclosures. Females survived better in E1 and E2, but not in E3. This could be due to the different timing of the translocations into the enclosures, since in E3 rabbits were released in May just after the peak of the breeding season. The accumulated stress of translocation plus depletion of body condition due to breeding (Kontsiotis et al., 2014) might have caused a reduction in female survival in that enclosure.

There was no detrimental effect of vaccination on short- or long-term rabbit survival (Fig. 1), but neither did we find clear support for beneficial effects. One possible explanation for why blind vaccination may have been ineffective for adult rabbits would be a high prevalence of natural antibodies against myxomatosis and RHD in the test population, analogous to what has been suggested for the RHD vaccination in the Iberian peninsula (Cotilla et al., 2010). Wild populations of rabbits are characterised by age-dependent increases in the prevalence of antibodies to myxomatosis and RHD (Calvete et al., 2002; Cooke et al., 2000), which may interfere with immunisation by vaccination, neutralising its effect on adult wild rabbit survival (Cabezas et al., 2006; Calvete et al., 2004a). Unfortunately, due to logistic constrains, we could not determine the immunological status of the donor population. However, the Andalusian Government released a public report in August 2012 showing that prevalence of antibodies against myxomatosis in 2009 was 59.3% and 61.4% for the Cordoba and Cadiz regions respectively (Junta-de-Andalucía, 2012). These values were similar to those obtained in the study site in 2009 by the new born animals in the enclosures for myxomatosis (~50% prevalence of antibodies) but slightly lower for RHD (~25% prevalence of antibodies) (Bertó-Moran et al., 2013). However, antibodies may not always be protective, especially in the case of RHD. For example, antibodies from non-pathogenic caliciviruses did not guarantee complete protection against RHD virus (Le Gall-Reculé et al., 2011). The same has been observed for RHD virus antibodies and the recently emerged variant RHDV2 (Le Gall-Reculé et al., 2013). However, it seems that infection with non-pathogenic caliciviruses does not cause significant interference with the diagnostic value of RHD infection using commercial enzyme-linked immunosorbent assay, cELISA (Zheng and Parkes, 2011). Moreover, such non-pathogenic viruses have

not been isolated yet in Spain. Therefore, in the absence of further information we could assume that most cases of RHD antibodies in Spanish wild rabbit reflect activity of virulent RHD virus (Cotilla et al., 2010).

Blood antibodies concentration can decrease naturally over time when there is absence of the viruses within a population (Mutze et al., 2002). This will suggest that apparently there is no long-term advantage of primary vaccination, and therefore it would be necessary to carry out periodic vaccination campaigns. Something difficult to achieve in the field because of the logistic and economics constrains. The time of vaccination is important (Guitton et al., 2008) and since the time of epidemics varies from one year to another, this would require the establishment of a health monitoring program of wild rabbit populations.

Outbreaks of myxomatosis and RHD occurred in our study enclosures between July-September 2009 and November 2009, respectively (Fig. 1S, see supplementary material). Based on the number of carcasses found during 2009, such outbreaks did not seem to be severe, which could partially explain the lack of significant increase in rabbit survival associated with double vaccination against these diseases.

Lack of effectiveness of double vaccination against myxomatosis and RHD is in agreement with two previous studies (Calvete et al., 2004a, 2004b). A considerable amount of information exists on the mechanisms through which vaccination against myxomatosis and RHD affects survival of wild translocated rabbits. In addition to the stress caused by the translocation process itself (e.g. capture, new area, etc.), the handling of vaccinated rabbits can be detrimental for survival especially for individuals in poor-condition (Cabezas et al., 2006) and juveniles (Calvete et al., 2004b). Vaccination campaigns in the field can additionally be influenced by the highly variable spatial-temporal pattern exhibited by the causative viruses (Villafuerte et al., 2000), which is a function of a panoply of factors such as the virulence of circulating strains or population density (Calvete, 2006; Ferreira et al., 2009), age (Calvete et al., 2004a, 2004b), timing of vaccination with respect to disease outbreaks (Calvete, 2006; Ferreira et al., 2009), body condition (Cabezas et al., 2006), and habitat management practices (Ferreira et al., 2014).

Our results suggest that care has to be taken before establishing a vaccination protocol in translocated rabbits against myxomatosis and RHD. In fact, the circumstances under which it is undertaken in the field by game managers and/or conservationists can be quite different between locations or seasons generating huge uncertainty in the possible outcomes. Given the impact of both diseases on rabbit survival, if it is not feasible to assess the immunological status of the donor population, the most conservative option is to carry out blind vaccination campaigns to ensure that translocated rabbits are at least protected against infection (or disease) after release, particularly if juveniles are being translocated since they generally have lower antibody prevalence (Guitton et al., 2008). However, particularly when adults are being moved as in the present study case, our results indicate that blind double vaccination may increase the economic cost without any benefit of significantly increased survival. We therefore recommend that game managers and conservationists carefully consider the cost/benefit trade-off of implementing vaccination as a management policy in rabbit translocations.

5. Conclusion

In particular, we recommend to assess the immunological status of the donor population prior to translocations since, if the prevalence of natural antibodies against these diseases is high, vaccination may be worthless especially when adult rabbits are moved. Such assessments should be performed just before the translocation, since natural antibody prevalence against myxomatosis and RHD varies independently among years, populations, ages and sexes (Arenas et al., 2012; Parkes et al., 2008; Santoro et al., 2014).

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References

Angulo, E., Villafuerte, R., 2003. Modelling hunting strategies for the conservation of wild rabbit populations. Biol. Conserv. 115, 291–301. doi:10.1016/S0006-3207(03)00148-4

Arenas, A.J., Napp, S., Arenas-Montes, A., Borge, C., Carbonero, A., Perea, A., Cadenas, R., García-Bocanegra, I., 2012. Serological response against myxoma virus and rabbit hemorrhagic disease virus in European wild rabbits using commercial vaccines. J. Wildl. Manage. 76, 102–107. doi:10.1002/jwmg.207

Bertó-Moran, A., Pacios, I., Serrano, E., Moreno, S., Rouco, C., 2013. Coccidian and Nematode Infections Influence Prevalence of Antibody To Myxoma and Rabbit Hemorrhagic Disease Viruses in European Rabbits. J. Wildl. Dis. 49, 10–17. doi:10.7589/2011-12-343

Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference. A practical information-theoretic approach. Springer-Verlag, Berlin.

Cabezas, S., Calvete, C., Moreno, S., 2006. Vaccination success and body condition in the European wild rabbit: applications for conservation strategies. J. Wildl. Manage. 70, 1125–1131. doi:10.2193/0022-541

Calvete, C., 2006. The use of immunization programs in wild populations: Modelling effectiveness of vaccination campaigns against rabbit hemorrhagic disease. Biol. Conserv. 130, 290–300. doi:10.1016/j.biocon.2005.12.025

Calvete, C., Estrada, R., Lucientes, J., Osacar, J.J., Villafuerte, R., 2004a. Effects of vaccination against viral haemorrhagic disease and myxomatosis on long-term mortality rates of European wild rabbits. Vet. Rec. 155, 388–92. doi:10.1136/vr.155.13.388

Calvete, C., Estrada, R., Osacar, J.J., Lucientes, J., Villafuerte, R., 2004b. Short-term negative effects of vaccination campaigns against myxomatosis and viral hemorrhagic disease (VHD) on the survival of european wild rabbits. J. Wildl. Manage. 68, 198–205. doi:10.2193/0022-541X(2004)068[0198:SNEOVC]2.0.CO;2

Calvete, C., Estrada, R., Villafuerte, R., Lucientes, J., Osácar, J.J., 2002. Epidemiology of viral hemorrhagic disease (VHD) and myxomatosis in the wild rabbit (Oryctolagus cuniculus) in the mid-Ebro valley, Spain. Vet. Rec. 150, 776–782.

Choquet, R., Reboulet, A.-M., Lebreton, J.-D., Gimenez, O., Pradel, R., 2005. U-CARE 2.2 User's Manual.

Cooke, B.D., Robinson, J.A., Merchant, J.C., Nardin, A., Capucci, L., 2000. Use of ELISAs in field studies of rabbit haemorrhagic disease (RHD) in Australia. Epidemiol. Infect. 124, 563–576.

Cotilla, I., Delibes-Mateos, M., Ramírez, E., Castro, F., Cooke, B.D., Villafuerte, R., 2010. Establishing a serological surveillance protocol for rabbit hemorrhagic disease by combining mathematical models and field data: Implication for rabbit

conservation. Eur. J. Wildl. Res. 56, 725–733. doi:10.1007/s10344-010-0368-y

Delibes-Mateos, M., Delibes, M., Ferreras, P., Villafuerte, R., 2008. Key role of European rabbits in the conservation of the western Mediterranean Basin hotspot. Conserv. Biol. 22, 1106–1117. doi:10.1111/j.1523-1739.2008.00993.x

Delibes-Mateos, M., Ferreira, C., Carro, F., Escudero, M. a, Gortázar, C., 2014. Ecosystem Effects of Variant Rabbit Hemorrhagic Disease Virus, Iberian Peninsula. Emerg. Infect. Dis. 20, 2166–2168. doi:10.3201/eid2012.140517

Delibes-Mateos, M., Ferreras, P., Villafuerte, R., 2009. European rabbit population trends and associated factors: A review of the situation in the Iberian Peninsula. Mamm. Rev. 39, 124–140. doi:10.1111/j.1365-2907.2009.00140.x

Ferreira, C., Ramírez, E., Castro, F., Ferreras, P., Alves, P.C., Redpath, S., Villafuerte, R., 2009. Field experimental vaccination campaigns against myxomatosis and their effectiveness in the wild. Vaccine 27, 6998–7002. doi:10.1016/j.vaccine.2009.09.075

Ferreira, C., Villafuerte, R., Villar, N., Castro, F., Ferreras, P., Rouco, C., Alves, P.C., de Reyna, L.A., Redpath, S., 2014. Experimental study on the effect of cover and vaccination on the survival of juvenile European rabbits. Popul. Ecol. 56, 195–202. doi:10.1007/s10144-013-0403-4

Guitton, J.S., Devillard, S., Guénézan, M., Fouchet, D., Pontier, D., Marchandeau, S., 2008. Vaccination of free-living juvenile wild rabbits (Oryctolagus cuniculus) against myxomatosis improved their survival. Prev. Vet. Med. 84, 1–10. doi:10.1016/j.prevetmed.2007.10.001

Junta-de-Andalucía, 2012. Programa de Vigilancia Epidemiológica de la Fauna Silvestre de Andalucía. (Report). Consejería de Medio Ambiente y Ordenación del territorio.

Le Gall-Reculé, G., Lavazza, A., Marchandeau, S., Bertagnoli, S., Zwingelstein, F., Cavadini, P., Martinelli, N., Lombardi, G., Guérin, J.L., Lemaitre, E., Decors, A., Boucher, S., Le Normand, B., Capucci, L., 2013. Emergence of a new lagovirus related to rabbit haemorrhagic disease virus. Vet. Res. 44, 1–13. doi:10.1186/1297-9716-44-81

Le Gall-Reculé, G., Zwingelstein, F., Fages, M.P., Bertagnoli, S., Gelfi, J., Aubineau, J., Roobrouck, A., Botti, G., Lavazza, A., Marchandeau, S., 2011. Characterisation of a non-pathogenic and non-protective infectious rabbit lagovirus related to RHDV. Virology 410, 395–402. doi:10.1016/j.virol.2010.12.001

Lebreton, J.-D., Burnham, K.P., Clobert, J., Anderson, D.R., 1992. Modelling Survival and Testing Biological Hypotheses Using Marked Animals: a Unified Approach with Case Studies. Ecol. Monogr. 62, 67–118.

Parkes, J.P., Glentworth, B., Sullivan, G., 2008. Changes in immunity to rabbit haemorrhagic disease virus, and in abundance and rates of increase of wild rabbits in Mackenzie Basin, New Zealand. Wildl. Res. 35, 775–779. doi:10.1071/WR08008

Rouco, C., Ferreras, P., Castro, F., Villafuerte, R., 2010. A longer confinement period favors European wild rabbit (Oryctolagus cuniculus) survival during soft releases in low-cover habitats. Eur. J. Wildl. Res. 56, 215–219. doi:10.1007/s10344-009-0305-0

Rouco, C., Ferreras, P., Castro, F., Villafuerte, R., 2008. The effect of exclusion of terrestrial predators on short-term survival of translocated European wild rabbits. Wildl. Res. 35, 625–632. doi:10.1071/WR07151

Santoro, S., Pacios, I., Moreno, S., Berto-Moran, A., Rouco, C., 2014. Multi-event capture-recapture modeling of host-pathogen dynamics among European rabbit

populations exposed to myxoma and rabbit hemorrhagic disease viruses: Common and heterogeneous patterns. Vet. Res. 45, 1–10.

Teixeira, C.P., de Azevedo, C.S., Mendl, M., Cipreste, C.F., Young, R.J., 2007. Revisiting translocation and reintroduction programmes: the importance of considering stress. Anim. Behav. 73, 1–13. doi:10.1016/j.anbehav.2006.06.002

Villafuerte, R., Calvete, C., Blanco, J.C., Lucientes, J., 1995. Incidence of viral hemorrhagic disease in wild rabbit populations in Spain. Mammalia 59, 651–659. doi:10.1515/mamm.1995.59.4.651

Villafuerte, R., Jordán, G., Angulo, E., 2000. Biología y factores de riesgo en el conejo silvestre.[Biology and risk factors for wild rabbit], in: Rossell, J.M. (Ed.), Enfermedades Del Conejo. Tomo II. Mundi-Prensa, Barcelona, Spain, p. 174–188.

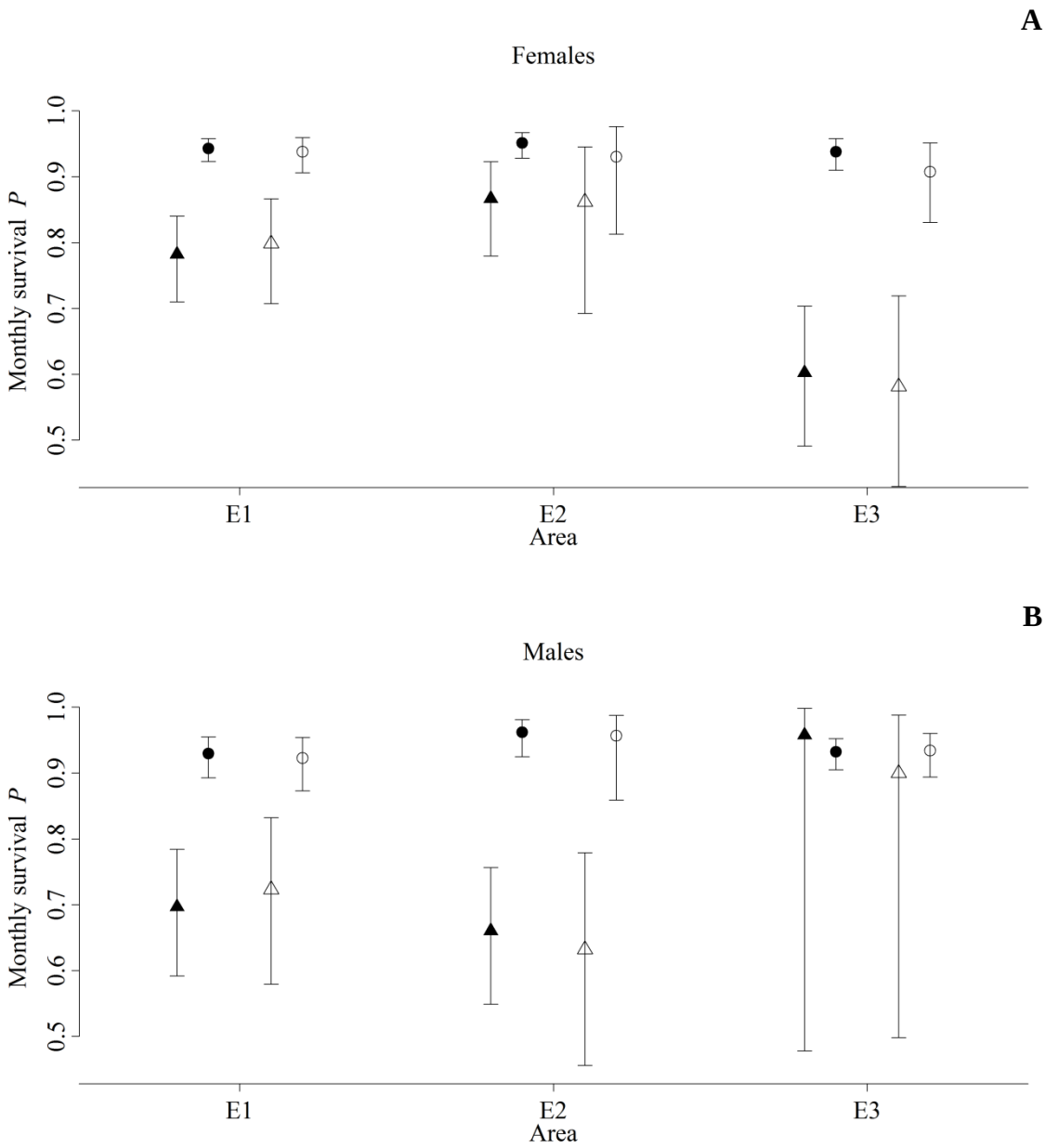
White, G.C., Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46. doi:10.1080/00063659909477239

Zheng, T., Parkes, J.P., 2011. Rabbit haemorrhagic disease: Advantages of cELISA in assessing immunity in wild rabbits (Oryctolagus cuniculus). Vet. Microbiol. 153, 387–392. doi:10.1016/j.vetmic.2011.05.049

Figure captions

Fig 1. Model averaged monthly survival estimates (±95% CI) in the three study areas (E1–3), of females (A) and males (B). Full and empty symbols indicate vaccinated and non-vaccinated rabbits, respectively. Triangles correspond to survival estimates in the interval just after release (i.e. short-term). Circles correspond to average survival through subsequent intervals (i.e. long-term).

FIGURE 1



Tables

Table 1. Ranking of possible models for rabbit survival based on the Aikaike Information Criterion corrected for small sample size.

Area	Model effects	AICc	ΔAICc	AICc w	Np	Dev
	sex* x *rel	**1278.26**	**0.00**	**0.47**	**13**	**307.34**
	sex x *(short x vac + long)*	1280.58	2.33	0.15	15	305.62
	sex x *(short + long* x *vac)*	1280.86	2.61	0.13	15	305.90
	Rel	1281.02	2.76	0.12	11	314.14
E1	*rel* x *vac*	1281.52	3.26	0.09	13	310.61
	sex x *rel* x *vac*	1282.73	4.47	0.05	17	303.72
	Sex	1316.26	38.01	0.00	11	349.39
	sex x *vac*	1320.20	41.94	0.00	13	349.29
	Constant	1322.45	44.19	0.00	10	357.60
	Vac	1324.46	46.20	0.00	11	357.59
	sex* x *rel	**778.61**	**0.00**	**0.53**	**13**	**304.94**
	sex* x *(short + long* x *vac)	**780.03**	**1.42**	**0.26**	**15**	**302.28**
	sex x *(short* x *vac + long)*	781.48	2.87	0.13	15	303.73
	sex x *rel* x *vac*	783.17	4.56	0.05	17	301.32
E2	*rel*	785.49	6.88	0.02	11	315.89
	rel x *vac*	786.07	7.46	0.01	13	312.40
	sex	798.06	19.45	0.00	11	328.46
	sex x *vac*	798.79	20.18	0.00	11	329.19
	vac	798.79	20.18	0.00	11	329.19
	constant	801.31	22.70	0.00	10	333.74
	sex* x *(short + long* x *vac)	**1587.11**	**0.00**	**0.40**	**15**	**476.03**
	sex* x *rel	**1587.75**	**0.64**	**0.29**	**13**	**480.76**
	sex* x *rel* x *vac	**1589.02**	**1.92**	**0.15**	**17**	**473.85**
	sex x *(short* x *vac + long)*	1589.11	2.01	0.15	15	478.04
E3	*rel*	1608.46	21.35	0.00	11	505.54
	rel x *vac*	1609.48	22.37	0.00	13	502.48
	sex x *vac*	1612.00	24.89	0.00	13	505.01
	sex	1613.32	26.21	0.00	11	510.40

vac	1620.90	33.80	0.00	11	517.98
constant	1621.77	34.67	0.00	10	520.89

All the final models shown here were parameterized according to previous model selection made on capture probability (see Methods for details). Accordingly, capture probability was hold as varying through capture sessions (*time*) in E1 and E2 and both through capture sessions and for each sex (*time* and *sex*) in E3. In bold are shown the more parsimonious models according to the correspondent AICc ranking (ΔAICc<2). Notation: *AICc*, Akaike information criterion corrected for small sample size; *ΔAICc*, the difference in AICc between the current model and the lowest AICc value; *AICc w*, Akaike's weight; np, number of estimable parameters; Dev, relative deviance; *sex*, sex; *short*, first interval after release, *long*, pooled intervals subsequent to the first capture session after release, *rel*, two periods corresponding to both *short* and *long*, and *vac*, vaccination status (vaccinated vs. unvaccinated).